

WHAT IS CLAIMED IS:

1. A method of detecting chemiluminescent emissions on a solid support,
the method comprising:

contacting a surface layer of the solid support with a composition
5 comprising a first chemiluminescent substrate capable of being activated by a first
enzyme to produce a first chemiluminescent signal;

detecting first chemiluminescent signal on the surface layer of the solid
support;

contacting the surface layer of the solid support with a composition
10 comprising a second chemiluminescent substrate capable of being activated by a
second enzyme to produce a second chemiluminescent signal; and

detecting second chemiluminescent signal on the surface layer of the solid
support;

wherein a plurality of probes are disposed in a plurality of discrete areas on
15 the surface layer at a density of at least 50 discrete areas per cm^2 , wherein at least
some of the probes are bound to a first enzyme conjugate comprising the first
enzyme, and wherein at least some of the probes are bound to a second enzyme
conjugate comprising the second enzyme.

2. The method of Claim 1, wherein the composition comprising the first
20 chemiluminescent substrate and the composition comprising the second
chemiluminescent substrate are contacted with the surface layer in the presence of
a composition comprising a chemiluminescent quantum yield enhancing material.

3. The method of Claim 1, wherein the first and second enzyme conjugates are each bound indirectly to a probe.

4. The method of Claim 3, wherein the first and second enzyme conjugates are bound to first and second target molecules, respectively, and wherein the first and second target molecules are each bound to a probe.

5. The method of Claim 4, wherein the first and second enzyme conjugates comprise antibody-enzyme conjugates and wherein the first and second target molecules comprises an antigen moiety capable of being bound by the antibody.

6. The method of Claim 1, wherein the first and second chemiluminescent substrates are 1,2-dioxetane substrates.

7. The method of Claim 1, wherein the first and second enzyme conjugates are each bound directly to probes.

8. The method of Claim 2, further comprising contacting the surface layer with the chemiluminescent quantum yield enhancing material before contacting the surface layer with the composition comprising the first chemiluminescent substrate.

9. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 100 discrete areas per cm^2 .

10. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 1,000 discrete areas per cm^2 .

11. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 25,000 discrete areas per cm^2 .

12. The method of Claim 1, wherein the density of discrete areas on the surface layer is at least 50,000 discrete areas per cm².

13. The method of Claim 1, further comprising:

contacting the support surface with a sample comprising first target
5 molecules labeled with a first label and second target molecules labeled with a second label prior to contacting the support surface with the substrate composition.

14. The method of Claim 13, wherein the first target molecules are labeled with the first enzyme to form the first enzyme conjugate and the second target molecules are labeled with the second enzyme to form the second enzyme
10 conjugate.

15. The method of Claim 13, wherein the first target molecules are labeled with a moiety capable of binding to the first enzyme conjugate and the second target molecules are labeled with a moiety capable of binding to the second enzyme conjugate.

16. The method of Claim 13, wherein the first target molecules comprise a first pool of target nucleic acids and wherein the second target molecules comprise a second pool of target nucleic acids.

17. The method of Claim 16, wherein the first and second pools of target nucleic acids each comprise mRNA transcripts of one or more genes or nucleic acids derived from mRNA transcripts of one or more genes.
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18. The method of Claim 16, wherein the first and second pools of target nucleic acids each comprise cDNA or cRNA derived from mRNA transcripts.

19. The method of Claim 17, wherein the concentration of the target nucleic acids in the first and second pools of target nucleic acids is proportional to the expression level of the genes encoding the target nucleic acid.

20. The method of Claim 1, wherein the first and the second
5 chemiluminescent substrates are each contacted with the surface layer in the presence of a chemiluminescent quantum yield enhancing material.

21. The method of Claim 1, wherein detecting the first chemiluminescent signal comprises determining the location on the support surface of the first chemiluminescent signal and wherein detecting the second chemiluminescent
10 signal comprises determining the location on the support surface of the second chemiluminescent signal.

22. The method of Claim 21, wherein control probes are located in one or more discrete areas on the support surface.

23. The method of Claim 22, wherein control probes are co-located in one
15 or more of the same discrete areas as the analyte probes.

24. The method of Claim 1, wherein the support surface further comprises fluorescent labels.

25. The method of Claim 1, wherein the first chemiluminescent signal and the second chemiluminescent signal have different emission maxima.

20 26. The method of Claim 25, wherein detecting the second chemiluminescent signal comprises:

filtering the emissions from the support surface with a filter adapted to reduce the intensity of the first chemiluminescent signal relative to the intensity of the second chemiluminescent signal; and

detecting the second chemiluminescent signal.

5 27. The method of Claim 1, wherein the first chemiluminescent signal and the second chemiluminescent signal have approximately the same emission maxima.

 28. The method of Claim 1, wherein the composition comprising the first chemiluminescent substrate and the composition comprising the second
10 chemiluminescent substrate are buffered compositions.

 29. The method of Claim 13, further comprising quantifying the amount of the first and the second target molecules in the sample.

 30. The method of Claim 29, wherein the support surface further comprises a fluorescent label and wherein quantifying comprises comparing the intensity of
15 the first and/or the second chemiluminescent signals to the intensity of the signal from the fluorescent label.

 31. The method of Claim 1, further comprising washing the surface layer of the solid support after incubating and before contacting the surface layer with the first substrate composition.

20 32. The method of Claim 1, further comprising washing the surface layer of the solid support after detecting the first chemiluminescent signal and before contacting the surface layer with the second chemiluminescent substrate composition.

33. The method of Claim 1, wherein the first and second chemiluminescent substrates are both 1,2-dioxetanes.

34. The method of Claim 1, wherein either of the first or second enzymes is β -galactosidase and the other enzyme is alkaline phosphatase.

5 35. The method of Claim 34, wherein the composition comprising the chemiluminescent substrate capable of being activated by the alkaline phosphatase enzyme is a 0.1 M solution of aminomethylpropanol and 1 mM MgCl_2 at a pH of 9.5.

10 36. The method of Claim 34, wherein the composition comprising the chemiluminescent substrate capable of being activated by the β -galactosidase enzyme is a 0.1 M solution of sodium phosphate and 1 mM MgCl_2 at a pH of 7.0.

37. The method of Claim 15, further comprising contacting the support surface with a composition comprising the first and second enzyme conjugates.

15 38. The method of Claim 37, wherein the first and second enzyme conjugates comprise enzyme-antibody conjugates and wherein the first and second target molecules are labeled with an antigen for the antibody.

39. The method of Claim 1, wherein the first chemiluminescent substrate is a 1,2-dioxetane substrate and the second chemiluminescent substrate is selected from the group consisting of an acridan ester substrate, an acridan thioester
20 substrate, an enol phosphate substrate, an acridan enol phosphate substrate, and a luminol substrate.

40. The method of Claim 2, wherein the chemiluminescent quantum yield enhancing material is an onium polymer selected from the group consisting of

poly(vinylbenzylammonium salts), poly(vinylbenzylphosphonium salts) and poly(vinylbenzylsulfonium salts).

41. The method of Claim 2, wherein the chemiluminescent quantum yield enhancing material is an onium copolymer.

5 42. The method of Claim 2, wherein the composition comprising the chemiluminescent quantum yield enhancing material further comprises an additive selected from the group consisting of BSA, cyclodextrins, negatively charged salts, alcohols, polyols, poly(2-ethyl-Z-oxazoline), zwitterionic surfactants, anionic surfactants, cationic surfactants, and neutral surfactants.

10 43. The method of Claim 2, wherein the composition comprising the chemiluminescent quantum yield enhancing material further comprises counterion moieties selected from the group consisting of halide, sulfate, alkylsulfonate, triflate, arylsulfonate, perchlorate, alkanoate, arylcarboxylate and combinations thereof.

15 44. The method of Claim 5, wherein the first or second enzyme conjugate is an antidigoxigenin:enzyme conjugate and wherein the corresponding target molecules are labeled with digoxigenin.

45. The method of Claim 44, wherein the target molecules labeled with digoxigenin comprise cDNA.

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